TECHNICAL MEMORANDUM

Tenmile Lakes Toxic Algae Monitoring, September 8th and 15th, 2009

Prepared for: **Tenmile Lakes Basin Partnership**

Prepared by: **Jacob Kann, Ph.D.**

Date: **September 18, 2009**

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The following results shows algal toxin results for September 8th and algal cell density results for September 15th, 2009 (see Figure 1 below for sample station location):

			Potentially Toxigenic Algal Species			Algal Toxins		
Station	Location	Date	*Microcystis* (cells/ml)	*Anabaena planktonica (cells/ml)*	*Aphanizomenon flos-aquae (cells/ml)*	Microcystin (µg/L)	Anatoxin -a (µg/L)	Saxitoxin (µg/L)
X	North Lake	9/8/2009	300,940	605,160	560,880	20	ND	ND
X	North Lake	9/15/2009	1,104,048	147,703	736,709	NT	NT	NT
Z	South Lake	9/15/2009	2,158,388	2,137	145,667	NT	NT	NT

World Health Organization Alert Level 1 increased vigilance guideline level is 500 cells ml^{-1} for potentially toxigenic species in drinking water systems.
World Health Organization Alert Level 2 public health posting guideline level is 2,000 cells ml^{-1} for potentially toxigenic species in drinking water systems
State of Oregon Recreational Guideline Levels is 40,000 cells/ml for *Microcystis* or 100,000 cells/ml for *Anabaena*
ND=not detect
NT=not tested

As noted in the previous toxic algal memo, patchy concentrations of blue-green algae were occurring in Tenmile Lakes on September 8th, and an additional sample was collected from North Tenmile at sample station "X" (see Figure 1 below). Results from this station showed both a high concentration of *Anabaena planctonica* (ABPL: 605,160 cell/ml) and *Microcystis aeruginosa* (MSAE: 300,940 cells/ml). The results shown above provide algal toxin results for the September 8th sample taken at station "X", as well as two follow-up samples for algal cell density taken at station "X" and a new station, station "Z" one week later on September 15th.

Algal toxin results (see lab reports below) show that the September 8th sample had 20 µg/L of microcystin, exceeding the 8 µg/L State of Oregon recreational posting guideline by 2.5x. These results confirm that MSAE levels exceeding 40,000 cells/ml can be associated with microcystin toxin levels that constitute a high probability of adverse health effects for recreational users of the lake (microcystin is a potent liver toxin associated with both chronic and acute health effects). Note that 2,000 cells/ml is the advisory level for using lake-water for potable purposes.

The above results also support statements in earlier memos that *Anabaena planktonica* (ABPL) has not been generally found to be associated with the algal toxin anatoxin-a (despite a cell density of >600,000 cells/ml, anatoxin-a was not detected). Likewise, although *Aphanizomenon flos-aquae* (APFA) has been associated with toxin production in other regions, the blooms typically experienced in Oregon have not been associated with toxin production; at a cell density of >560,000 cells/ml neither anatoxin nor saxitoxin was detected in the Tenmile sample.

A sample collected one week later at station "X" continued to show prevalent blue-green algae, including a 3.6 fold increase in MSAE (1,104,048 cells/ml). An additional sample from South Tenmile also showed a very high density of MSAE (2,158,388 cells/ml) and was the predominant species at that station (see lab report below). ABPL and APFA also comprised the bloom at both stations. Photos of these areas clearly show the high concentrations of cyanobacteria (blue-green algae) at these locations (Figure 2).

These results show that blue-green blooms are prevalent in varying areas of both North and South Tenmile Lakes and that both cell density and microcystin toxin results exceed guidelines for recreation posting. In addition, given varying effectiveness of home-owner drinking water treatment systems and the prevalence of toxic blooms, I would advise against utilizing lake water for potable purposes at his time.

Due to the patchy nature of blue-green algal blooms it is possible for higher Microcystis aeruginosa and Anabaena flos-aquae densities (and therefore higher microcystin toxin and anatoxin concentrations to be present in areas not sampled in this survey, particularly along shorelines or during calm conditions of little to no wind. Given the lakes' demonstrated history of toxic blooms, and the fact that all areas of the lake cannot be tested at all times, those utilizing the lake for drinking water should <u>always</u> follow Oregon Health Division recommendations for purification (attached). In addition, recreational users should <u>always</u> avoid contact with water whenever noticeable surface concentrations of algae are evident or when the lake has an obvious green to blue-green appearance. Moreover, because pets or other domestic animals are the most likely to ingest contaminated water, these animals should not be allowed access to the lakeshore whenever either noticeable surface concentrations of algae or an obvious green to blue-green appearance is evident.

References for Alert Levels

Carey, C.C., and J.F. Haney, and K.L. Cottingham. 2007. First report of microcystin-LR in the cyanobacterium *Gloeotrichia echinulata.* Environmental toxicology 22:337-339.

Kann, J. 2007. Tenmile Lakes Toxic Algal Sampling Program: 2006 Data Summary Report. Tenmile Lakes Basin Partnership, Lakeside OR 97520

Falconer et al. 1999. Safe levels and safe practices. Pages 155-177 *in*: I. Chorus and J. Bartram, editors. *Toxic Cyanobacteria in water: a guide to their public health consequences*. World Health Organization Report. E & FN Spon, London and New York.

Stone, D., and W. Bress. 2007. Addressing Public Health Risks for Cyanobacteria in Recreational Freshwaters: The Oregon and Vermont Framework. Integrated Environmental Assesment and Managment; 3(1): 137 - 143 (2007). http://www.oregon.gov/DHS/ph/hab/docs/Stone_cyano_rec.pdf

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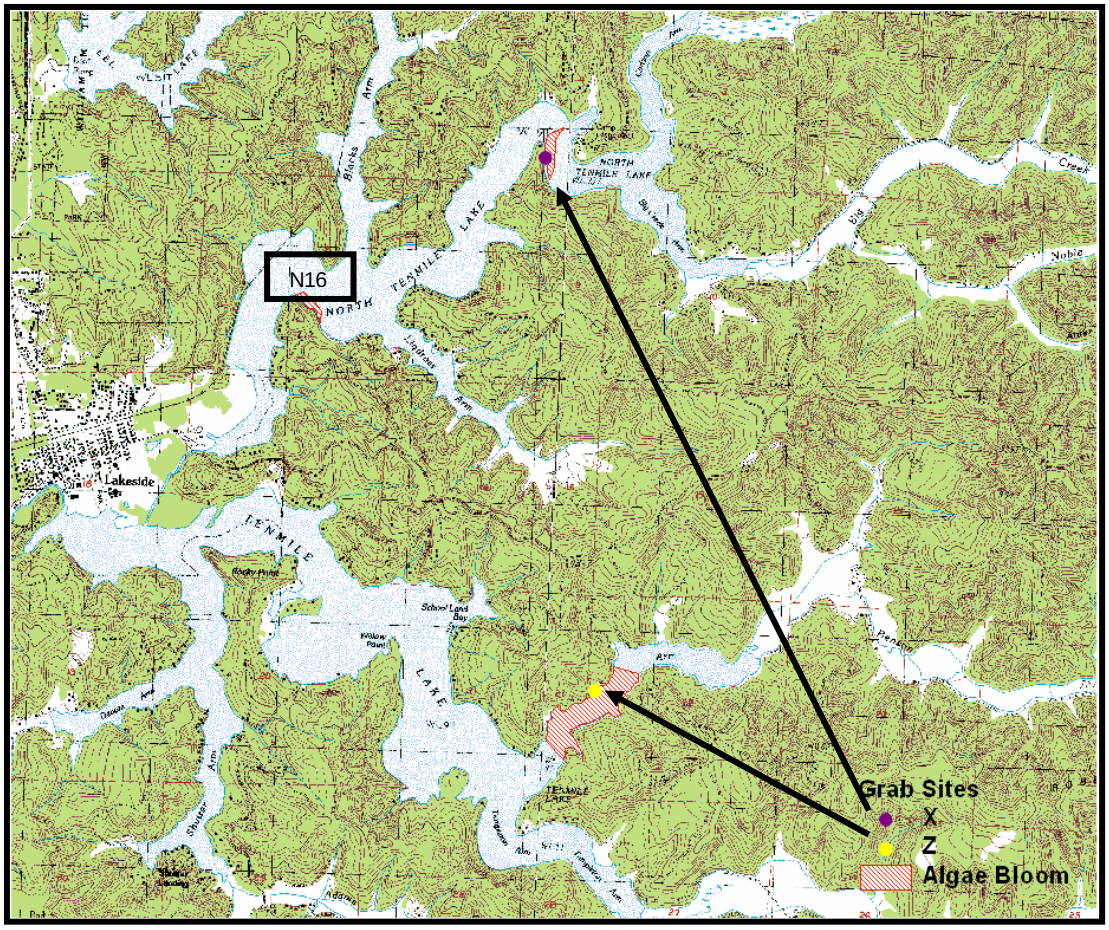

Figure1. September 15 sample station and bloom location for potentially toxigenic cyanobacteria, Tenmile Lakes, 2009.

Figure 3. September 15th bloom conditions at varying locations Tenmile Lake (see map above).

Appendix I: Aquatic Analysts Phytoplankton Lab Sheets

Phytoplankton Sample Analysis

Sample:	Tenmile Lake
Sample Site:	X
Sample Depth:	
Sample Date:	15-Sep-09
Total Density (#/mL):	50,963
Total Biovolume (um^3/mL):	81,747,133
Trophic State Index:	81.6

	Species	Density #/mL	Density Percent	Biovolume um^3/mL	Biovolume Percent	Group
1	Aphanizomenon flos-aquae	40,590	79.6	46,029,060	56.3	bluegreen
2	Anabaena planctonica	5,187	10.2	26,575,626	32.5	bluegreen
3	Microcystis aeruginosa	4,059	8.0	8,832,384	10.8	bluegreen
4	Chlamydomonas sp.	902	1.8	293,150	0.4	green
5	Oocystis sp.	226	0.4	16,913	0.0	green

Aphanizomenon flos-aquae cells/mL =	730,620
Aphanizomenon flos-aquae heterocysts/mL =	6,089
Anabaena planctonica cells/mL =	145,222
Anabaena planctonica heterocysts/mL =	2,481
Microcystis aeruginosa cells/mL =	1,104,048

Note: 200 count.

Aquatic Analysts **Sample ID:** MB69

Phytoplankton Sample Analysis

Sample:	Tenmile Lake
Sample Site:	Z
Sample Depth:	
Sample Date:	15-Sep-09
Total Density (#/mL):	22,123
Total Biovolume (um^3/mL):	26,791,217
Trophic State Index:	73.6

	Species	Density #/mL	Density Percent	Biovolume um^3/mL	Biovolume Percent	Group
1	Aphanizomenon flos-aquae	17,955	81.2	9,049,069	33.8	bluegreen
2	Microcystis aeruginosa	3,847	17.4	17,267,101	64.5	bluegreen
3	Anabaena planctonica	107	0.5	391,152	1.5	bluegreen
4	Chlamydomonas sp.	107	0.5	34,733	0.1	green
5	Cocconeis placentula	107	0.5	49,161	0.2	diatom

Aphanizomenon flos-aquae cells/mL =	143,636
Aphanizomenon flos-aquae heterocysts/mL =	2,031
Anabaena planctonica cells/mL =	2,137
Microcystis aeruginosa cells/mL =	2,158,388

Note: 200 count.

Aquatic Analysts **Sample ID:** MB70

Appendix II: GreenWater Labs Algal Toxin Results

GreenWater laboratories — aquatic analysis ... research ... consulting

Anatoxin-a, Microcystin and Saxitoxin Analysis Report
Project: TLBP
(Tenmile – North Lake)

Sample Identification	Sample Collection Date
Tenmile - North Lake	9/8/09

Toxin – Anatoxin-a (ANTX-A), microcystin (MC), saxitoxin (STX)

Sample Prep – The sample was ultra-sonicated to lyse cells and release toxins. Solid phase extraction (SPE) was also utilized for ANTX-A prep and preconcentration (100x). Duplicate samples were spiked with 0.1 µg/L of ANTX-A and 0.5 µg/L STX, which provided quantitative capability and additional qualitative confirmation.

Analytical Methodology – Liquid chromatography/ mass spectrometry/ mass spectrometry (LC/MS/MS) was utilized for the determination of ANTX-A. The $[M+H]^+$ ion for ANTX-A (*m/z* 166) was fragmented and the major product ions (*m/z* 149, 131, 107, and 91) provided both specificity and sensitivity. The current methodology established a detection limit of 0.05 µg/L and a quantification limit of 0.1 µg/L for ANTX-A.

A microcystins enzyme linked immunosorbent assay (ELISA) was utilized for the quantitative and sensitive congener-independent detection of MCs. The current assay is sensitive to down to a detection/quantification limit of 0.15 µg/L for total MCs.

A saxitoxin enzyme linked immunosorbent assay (ELISA) was utilized for the quantitative detection of saxitoxin. The current assay is sensitive down to a detection/quantification limit of 0.1 µg/L saxitoxin.

Summary of ANTX-A/MC/STX Results

Sample	ANTX-A level (µg/L)	MC level (µg/L)	STX level (µg/L)
Tenmile – North Lake	ND	≈ 20	ND

ND = Not detected above the detection limit
Detection Limit = 0.05 µg/L (ANTX-A), 0.15 µg/L (MC), 0.1 µg/L (STX)
Limit of Quantification = 0.1 µg/L (ANTX-A & STX), 0.15 µg/L (MC)

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Tenmile Lake Basin Partnership

SAXITOXIN RESULTS

Tested on: 9/16/2009
Method: Enzyme-Linked ImmunoSorbent Assay (ELISA)
Analyte: Saxitoxins
Analyzed by: Amanda Foss

Sample ID/ Date Collected	Initial Conc. Factor	Dilution Ratio	Assay Value, ug/L	Final Dilution Factor	Avg. Std. Recovery(%)	Avg. Spike Recovery(%)	Final Concentration (ug/L)	Average (ug/L)
Tenmile North Lake	1x	none	ND	1	99	96	ND	ND
9/8/2009	1x	none	ND	1	99	96	ND	

ND = Not detected above Quantification limit
Quantification limit = 0.10 µg/L
Standard = 0.5 µg/L STX
Sample spike = 0.5 µg/L STX

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Tenmile Lake Basin Partnership

MICROCYSTIN RESULTS

Tested on: 9/16/2009
Method: Enzyme-Linked ImmunoSorbent Assay (ELISA)
Analyte: Microcystins
Analyzed by: Amanda Foss

Sample ID/ Date Collected	Initial Conc. Factor	Dilution Ratio	Assay Value, ug/L	Final Dilution Factor	Avg. Std. Recovery(%)	Final Concentration (ug/L)	Average (ug/L)
Tenmile North Lake	1x	1:10	1.97	10	100	19.7	20
9/8/2009	1x	1:10	2.01	10	100	20.1	
		1:100	0.20	100	100	20.0	
		1:100	0.20	100	100	20.0	

ND = Not detected above Quantification limit
Quantification limit = 0.15 µg/L
Standard = 1 µg/L MCLR
Sample spike = 1 µg/L MCLR

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O**regon Health Division**
Drinking water treatment guidance
August 31, 2001

DHS Contact Information:

Harmful Algae Program Coordinator: Laura Boswell at (971) 673 – 0438
If she is not available call the main line for the Office of Environmental
Public Health at: (971) 673 – 0440 or
Toll Free: (877) 290 – 6767 and press 0. Ask for Laura Boswell

1. Treatment systems should consist of sand filtration followed by chlorination, followed by activated charcoal filtration. It is essential that sand filtration be done before disinfection to remove as many algal cells as possible without killing or rupturing them.

2. Chlorination systems should be capable of maintaining at least 1 ppm of chlorine residual for at least 20 minutes contact time before the water enters the activated charcoal system.

3. The final step in the process should be effective activated charcoal treatment to remove toxin remaining after the sand filtration and disinfection processes.

4. All treatment equipment used should meet NSF standard 53, and should be adequately sized to treat the maximum amount of water that you use. Treatment equipment needs regular monitoring and servicing to assure that it functions properly.

5. Ideally all water entering your home should be treated as recommended. It is possible to treat only water used in the kitchen, but this increases chances that animals or pets would inadvertently drink untreated water.

As more monitoring is done and toxin levels are measured this advisory may be altered. The advisory is to remain in effect until specifically changed or lifted by county and state health officials.

FACT SHEET

TOXIC *MICROCYSTIS* BLOOMS IN TENMILE LAKES

(information modified from Oregon Health Division Document: Hazards from *Microcystis aeruginosa* in Fresh Water – http://www.ohd.hr.state.or.us/esc/docs/mafact.htm)

➢ What is a toxic bloom of *Microcystis aeruginosa*?

Microcystis aeruginosa is a species of blue-green algae that grows naturally in many surface waters. In most bodies of fresh water and most weather conditions it does not pose a hazard to wildlife or human beings. However, under certain conditions (such as when the water is warm with abundant nutrients) *Microcystis aeruginosa* can grow more rapidly than normal. The result can be excessive numbers of large colonies that form floating masses on the water surface or that are dispersed within the water column. These occurrences are called "algal blooms". *Microcystis aeruginosa* can produce natural toxins (called microcystins) that are very potent, and these toxins are higher in concentration during bloom conditions. The microcystin toxins are produced and contained inside the *Microcystis* cells, and are released to the water when the cells die and disintegrate. Also, since the cells are very small, they can be ingested along with the water. Toxin levels in a water body tend to be higher near shorelines and at the surface of the water where animal and human contact is most likely.

➢ What are the primary toxic effects of these blooms?

The primary toxic effect of microcystins is on the liver. At very high doses, death of liver cells and destruction of blood vessels in the liver can result in serious injury and possibly death. Though less is known about the long-term effects of microcystin toxins, animal studies have shown these toxins can cause chronic liver damage and may promote the formation of liver tumors. These effects are more likely to occur if exposure is frequent over a long period of time.

The levels of toxin necessary to produce immediate or acute illness in humans and animals are much higher than levels that may cause chronic liver injury. Drinking water standards are usually based on chronic effects. Currently, there is no drinking water standard in the U.S. for microcystins. Canada, Australia, and Great Britain have developed a guideline level of 1 microgram toxin per liter of water, or 1 part per billion (1 ppb). During algal blooms, toxin levels can greatly exceed 1 ppb.

➢ How is it determined when the water becomes safe once a bloom is reported?

Changes in weather or in other conditions in a water body influence the growth of blue-green algae. Generally, cooler weather, rainfall, and reduced sunshine will lead to reductions in algal growth and toxin levels. Algal blooms generally peak and die off rapidly and toxin levels in the water decline over days or weeks. Only blue-green algae experts can distinguish visually between different kinds of algal growth, and are able to determine when blooms have disappeared. Testing of the water is the only way to be certain that toxin levels are no longer dangerous.

➢ When does the Oregon Health Division Issue Warnings?

Drinking Water -- When measured or estimated toxin levels reach 1 ug/l the Department of Human Services, Office of Public Health Systems issues public advisories or warnings. These will include warnings regarding the use of water for drinking or food preparation unless the water has been treated following specific guidelines for destroying and removing toxins. Animals should be kept away from water during periods when microcystin toxin levels exceed 1 ug/l, because drinking the water can cause serious or even fatal illness.

Contact Recreation -- If levels are high enough to pose hazards for swimming, water-skiing or other direct skin contact activities, the advisories will warn against water contact. Generally skin hazards occur where the water has a green or blue-green color or where there are visible clumps or mats of algae present in the water. When measured toxin levels reach 5 ug/L or cell counts reach 15,000 cells/ml, contact recreation is considered unsafe.

➢ Can testing ensure that all areas of the lake are safe?

No, due to the patchy nature of blue-green algal blooms it is possible for higher *Microcystis* densities (and therefore higher microcystin toxin concentrations) to be present in areas not sampled in a given survey, particularly along shorelines or during calm conditions of little to no wind. **Therefore, when a lake has a demonstrated history of algal toxicity or the presence of known toxin producing algal species, those utilizing the lake for drinking water should always follow Oregon Health Division recommendations for purification. In addition, recreational users should always avoid contact with water whenever noticeable surface concentrations of algae are evident or when the lake has an obvious green to blue-green appearance.**

➢ Are domestic animals at risk during blooms?

Yes, pets or other domestic animals are the most likely to ingest contaminated water, these animals should not be allowed access to the lakeshore whenever either noticeable surface concentrations of algae or an obvious green to blue-green appearance is evident.

➢ Is it safe to eat fish and other aquatic life?

Clams, mussels, snails and other shellfish should not be eaten during microcystin advisory periods, but it is believed that fish can be safely eaten if they are cleaned and all internal organs discarded. Internal organs of such fish may be toxic even to animals.

➢ How much does testing cost?

Samples must be shipped to qualified laboratories for analysis. A microscopic determination to quantify the number of Microcystis colonies and cells costs $90 per sample. A specialized test to analyze for the microcystin toxin concentration costs $100 per sample (overnight shipping costs not included), and for anatoxins the cost is $250/sample .

- NOTE: A fact sheet about microcystin toxin and its effects may be found on the Web at www.dhs.state.or.us/publichealth/esc/docs/mafact.cfm